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Mammosomatotroph Cell Adenoma of the Human Pituitary: A Morphologic Entity

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Summary. Nine cases of a hitherto undescribed morphologic entity, termed mammosomatotroph cell adenoma of the human pituitary, are reported. These tumors, occurring mostly in men, are invariably associated with acromegaly (or gigantism) and high-normal or slightly elevated blood prolactin levels, and it cannot be distinguished clinically from well-differentiated growth hormone cell or mixed growth hormone cell-prolactin cell adenomas. They show a slow growth rate and usually exhibit a diffuse pattern and intense cytoplasmic acidophilia by histology. The immunoperoxidase technique detects both growth hormone and prolactin within the same cells. Electron microscopy reveals monomorphous tumors with a fine structure markedly similar to that of well-differentiated, densely granulated growth hormone cell adenomas. An added feature and diagnostic marker of mammosomatotroph cell adenoma is the presence of extracellular deposits of secretory material. One tumor shows a marked abnormality of hormone packaging and storage, resulting in the cytoplasmic accumulation of pleomorphic bodies containing semicrystalline secretory material.

Key words: Pituitary adenoma – Ultrastructure – Immunocytochemistry – Acromegaly – Hyperprolactinemia

Introduction

Landolt and Rothenbühler (1978) described 3 monomorphous pituitary adenomas associated with acromegaly which, by electron microscopy, contained large extracellular deposits of secretory material. Since these aggregates reacted positively for growth hormone by immunoelectron microscopy, Landolt and Rothenbühler (1978) considered the tumor type as a rare variant of growth hormone cell adenomas. They made no attempt to demonstrate prolactin in the tumor, although one of their patients was known to have markedly elevated blood prolactin

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levels. Adenomas displaying similar ultrastructural features have been sporadically observed in other series as well (Kameya et al. 1980; Trouillas et al. 1980).

In our material of 610 surgically-removed pituitary adenomas investigated by histology, immunocytochemistry and electron microscopy, the presence of exocytoses was invariably associated with production of prolactin by the tumor, even if blood prolactin levels were not substantially elevated. Therefore, it was reasonable to assume that growth hormone-secreting tumors, containing extracellular deposits of secretory material, are capable of concomitant production of prolactin as well. The histological, immunocytochemical and ultrastructural features of 9 such tumors will be reported here.

Material and Methods

Patient Material

Of 610 surgically-removed pituitary adenomas, 330 (54%) derived from cells of the "acidophil" cell line. Ninety-eight tumors consisted solely of growth hormone cells, 176 were prolactin cell adenomas, 28 tumors represented true mixed adenomas composed of growth hormone cells and prolactin cells, and 19 immature monomorphus neoplasms, usually containing both growth hormone and prolactin, were diagnosed as acidophil stem cell adenomas. Based on their immunocytochemical and fine structural features, the 9 remaining tumors, removed from 8 acromegalic men and 1 woman, with the average age of 45.9 years, were termed mammosomatotroph cell adenomas.

Morphologic Techniques

Pieces of tumor tissue had been fixed immediately after their removal. For histology and immunocytochemistry, formalin-fixed (10% buffered formalin), paraffin-embedded tissue was used. Paraffin sections of 4–6 μm thickness were stained routinely with hematoxylin-phloxin-saffron and PAS. The immunoperoxidase technique was performed, as described elsewhere (Kovacs et al. 1981), on paraffin sections using antihuman growth hormone (Imperial Cancer Research Foundation, London, England) and antihuman prolactin (donated by Dr. H. Friesen, Department of Physiology, University of Manitoba, Winnipeg, Man., Canada) as primary antibodies. The specificity of immunostaining was verified by 1) increasing dilution of primary antibody; 2) saturation of primary antibodies with their corresponding antigen; 3) omission of primary antibody and its replacement either by normal serum or phosphate buffered saline.

For electron microscopy, tissue was fixed in 2.5% glutaraldehyde in Sorensen's buffer, postfixed in 1% OsO_4 in Millonig's buffer, dehydrated in graded ethanol and embedded in Epon 812 or Epon-Araldite mixture. In selecting tissue for fine structural study, semithin sections were cut and stained with toluidin blue. The ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

For immunoelectron microscopy, pieces of tissue from 6 of the 9 cases were fixed in 2.5% glutaraldehyde in Sorensen's buffer, dehydrated without previous osmification in graded ethanol and embedded in Epon 812 or Epon-Araldite mixture. The immunoperoxidase technique for growth hormone and prolactin was performed by the method of Moriarty and Garner (1977).

Results

Clinical Findings. The most important clinical and laboratory findings are summarized in Table 1. All patients had typical acromegaly. In the cases

it was recorded, blood prolactin was high-normal or mildly elevated. The only exception was the large, invasive tumor in case 7 which produced growth hormone as well as large amounts of prolactin. Despite the sometimes very long (25 years) history of acromegaly, the majority of tumors were well circumscribed intrasellar adenomas. The postoperative blood growth hormone values were recorded within the normal range in 7 patients.

Morphologic Findings

By histology, all 9 adenomas exhibited a similar diffuse growth pattern and most of them showed intense cytoplasmic acidophilia. Only 3 neoplasms were composed of an admixture of densely and sparsely granulated ("chromophobic") cells. The adenomas were PAS negative, showed no pleomorphism and mitoses were not apparent.

By the *immunoperoxidase technique,* a well-defined positivity for immunoreactive growth hormone was attained in all cases tested (Fig. 1). With the exception of the tumor in case 7, the immune precipitate appeared to be present in every cell and was detected either over the entire cytoplasm or in the Golgi region, depending on the granularity of adenoma cells. At the light microscopic level, the immunostaining for prolactin was equivocal in cases 1 and 2, showing only light positivity at the periphery of the cells. In the other cases, however, well-defined positivity for immunoreactive prolactin was demonstrated. The percentage of prolactin-containing cells varied not only from case to case but also in different areas of the same adenoma.

Electron microscopy revealed well differentiated adenomas in all 9 cases. Seven tumors resembled well-granulated growth hormone cell adenomas composed of closely apposed polyhedral cells, possessing fairly uniform nuclei with light chromatin. Spherical, dense, moderately prominent nucleoli were frequently noted. In the electron lucent cytoplasm, the well developed RER was represented by numerous slim cisternae. The large Golgi complexes regularly contained forming secretory granules. A varying number of fibrous bodies in association with some tubular SER, occurred in all tumors. The spherical or oval mitochondria were present in fair number, possessed lamellar cristae and light or moderately dense granular matrix.

The secretory granules measured between 150–2,000 nm. The tumors in cases 2, 6 and 9, were extremely densely granulated, containing chiefly large (over 450 nm) secretory granules. In the other adenomas, numerous cells possessed smaller (150–450 nm) and sometimes less secretory granules. With the exception of the tumor in case 4, the adenomas contained two types of secretory granules. Most of them were spherical or oval and evenly electron dense with tightly fitted, practically indiscernible limiting membrane, similar to those seen in densely granulated growth hormone cell adenomas. The minority had irregular, frequently mottled core and a well visible halo under the finely ruffled limiting membrane. The latter type often appeared to be involved in the process of granule extrusion. The extruded lumps of secretory material, present in the intercellular space or

Table 1

Patient, Age, Sex	Major clinical and X-ray findings		Hormone levels	Estimated tumor size[a]	Histology	Immunohistochemistry	
						GH	PRL
1. 31 years, M	Acromegaly (2 years). x-ray: enlarged sella with double floor and sloping to the right. No erosion, no suprasellar growth.	preop.	GH: 32 ng/ml insulin → 32 ng/ml PRL: 8.5 ng/ml TRH → 19.1 ng/ml	Grade II-O	partly acidophilic, partly chromophobic	+ + +	±
		postop.	GH: 2.0 ng/ml insulin → 31.5 ng/ml PRL: 7.0 ng/ml TRH → 34.0 ng/ml				
2. 56 years, M	Acromegaly (> 25 years). Diabetes mellitus. x-ray: enlarged sella with depression of floor. No erosion, no suprasellar extension. Previous radiotherapy and bromocriptine treatment.	preop.	GH: up to 100 ng/ml PRL: 7.5 ng/ml TRH → 20.6 ng/ml	Grade II-O	acidophilic	+ + +	±
		postop.	GH: 3.0–2.0 ng/ml (random) PRL: 7.5 ng/ml (random)				
3. 40 years, M	Incidental discovery of acromegaly. x-ray: enlarged sella with double floor and slope to the left. Localized erosion. No suprasellar extension.	preop.	GH: 64 ng/ml insulin → 70 ng/ml PRL: 17 ng/ml TRH → 30 ng/ml	Grade III-O	partly acidophilic, partly chromophobic	+ +	+ +
		postop.	GH: 5 ng/ml insulin → 26 ng/ml PRL: 9 ng/ml TRH → 25 ng/ml				
4. 61 years, M	Acromegaly (8–10 years). x-ray: slightly enlarged sella with double contour of floor. No suprasellar extension.	preop.	GH: 15 ng/ml insulin → 53 ng/ml (exaggerated reponse) GH: 20 ng/ml TRH → 74 ng/ml (anomalous rise) PRL: 27 ng/ml TRH → 30 ng/ml	Grade II-O	acidophilic	+ + +	+ +
		postop.	GH: 4.0 ng/ml insulin → 13 ng/ml PRL: 4.0 ng/ml TRH → 10 ng/ml				

5. 39 years, M	Incidental discovery of acromegaly. x-ray: enlarged sella.	preop.	GH: 21 ng/ml PRL: 46 ng/ml	Grade II-O	acidophilic	++	+
		postop.	GH, PRL: within normal limits				
6. 55 years, M	Acromegaly (7 years). x-ray: enlarged sella with localized erosion. No suprasellar growth	preop.	GH: >50 ng/ml PRL: 14 ng/ml	Grade III-O	acidophilic	+++	+
		postop.	not available				
7. 29 years, M	Acromegaly (insidious onset), hypogonadism (pre-pubertal), blurred vision (2 years). x-ray: markedly enlarged sella with extensive erosion. Sellar mass with supra and infrasellar extension	preop.	GH: 18 ng/ml insulin → 58.5 ng/ml PRL: 4,840 ng/ml TRH → 6,950 ng/ml Testosterone: <100 ng/ml	Grade IV-C	partly acidophilic, partly chromophobic	+	+++
		postop.	GH: 8 ng/ml insulin → 23 ng/ml PRL: 2,200 ng/ml TRH → 4,010 ng/ml				
8. 57 years, F	Progressing acromegaly (4 years)	preop.	GH: >75 ng/ml PRL: not recorded	Grade III-O	acidophilic	+++	++
		postop.	GH: <1 ng/ml PRL: not recorded				
9. 45 years, M	Acromegaly (>25 years). x-ray: enlarged sella with no erosion. CT scan: no suprasellar extension.	preop.	GH: 60 ng/ml insulin → 143 ng/ml PRL: 29 ng/ml TRH → 31.6 ng/ml	Grade II-O	acidophilic	insufficient specimen	
		postop.	GH: 3.1 ng/ml PRL: 8.8 ng/ml				

[a] The neuro-radiological classification of Hardy, J. (Transphenoidal surgery of hypersecreting pituitary tumors. In: Diagnosis and treatment of pituitary tumors. Internat Congr Series No. 303, Excerpta Medica, Amsterdam, 1973, pp. 179–194) is used

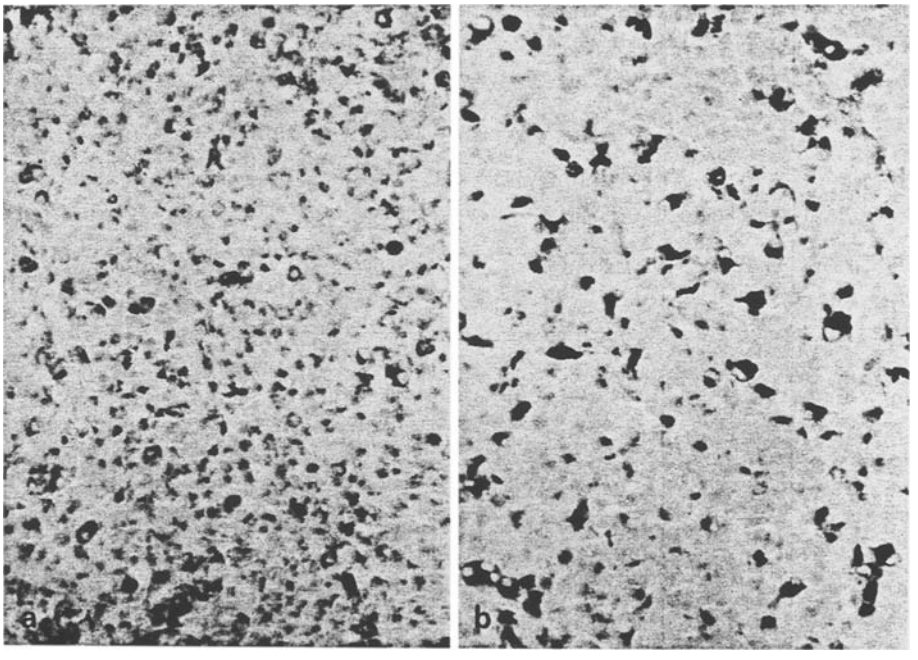

Fig. 1 a, b. Immunoreactive growth hormone is present in practically every cell of this mammosomatotroph cell adenoma **a**. In the same tumor, prolactin is detectable in many, but not all cells. As indicated by the staining pattern, some prolactin-containing cells are densely granulated **b**. Case 3; Immunoperoxidase technique for growth hormone and prolactin, ×250

at the perivascular surface of adenoma cells, retained a high electron density, as opposed to exocytoses in prolactin cell adenomas (Fig. 2). The formation of 25–100 nm wide channels between secretory granules and the extracellular space, funneling secretory material, was observed in all cases (Fig. 3). This appears to be a unique feature of this adenoma type.

In the adenoma of case 4, a marked abnormality of formation and storage of secretory material was observed (Fig. 4). The cells, exhibiting the same well differentiated features as those of other tumors in this series, possessed only a limited number of spherical or slightly oval secretory granules measuring 200–450 nm. Most of the secretory material was present within strikingly pleomorphic membranebound bodies measuring up to 2,000 nm or more in their largest diameter. Many of these showed signs of growing only to certain privileged directions, indicating crystallization within their substance (Fig. 5). Some of the needleshaped crystalline structures reached 4,000 nm in length. Numerous pleomorphic secretory bodies with mottled or flaky material emptied their contents into the extracellular space.

Immunoelectron microscopy for growth hormone and prolactin was performed in cases 2–7. In adenomas 2–6, every tumor cell contained a varying number of secretory granules exhibiting well defined positivity for immunoreactive growth hormone (Fig. 6). With the exception of case 7, the granules

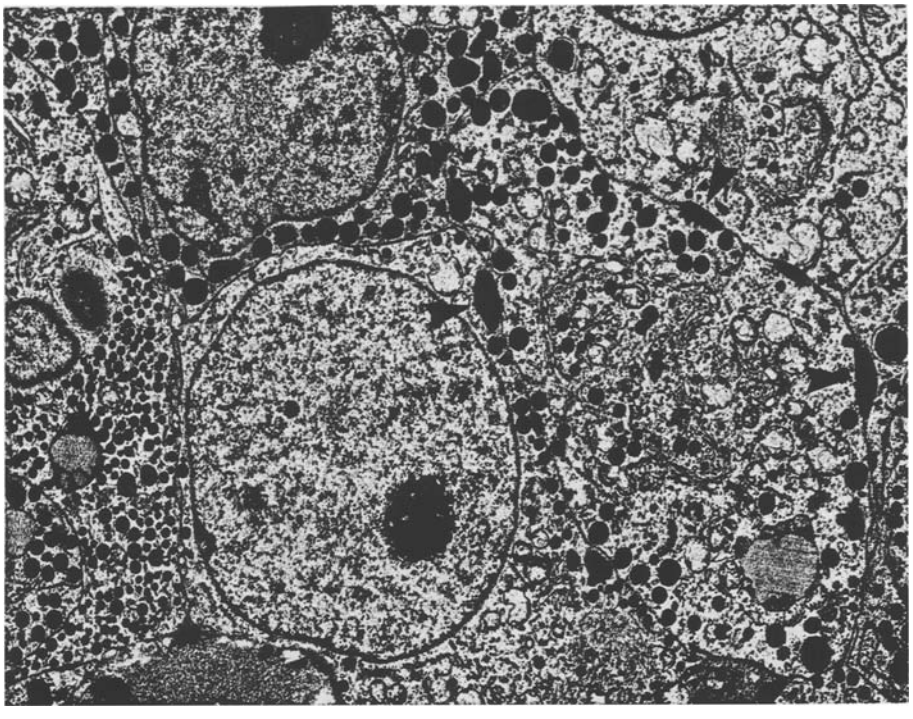

Fig. 2. The fine structure of this tumor is similar to that of densely granulated growth hormone cell adenomas. Some secretory granules are, however, unusually large, and extracellular deposits of secretory material are also noted (*arrowheads*). Case 2, × 5,200

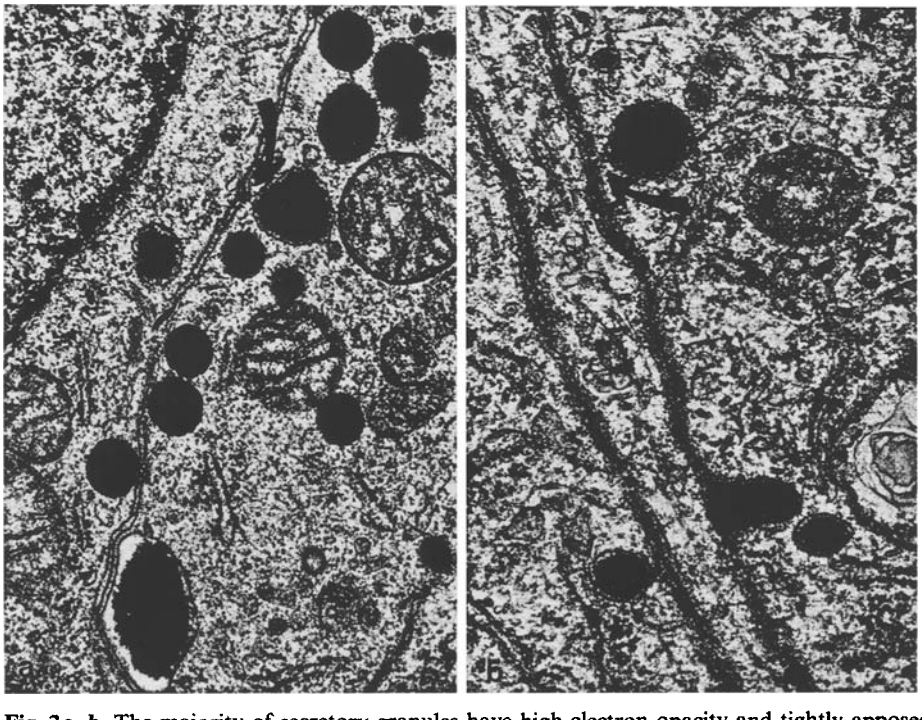

Fig. 3a, b. The majority of secretory granules have high electron opacity and tightly apposed limiting membranes. Others have a mottled, often irregular core with a prominent halo under the limiting membrane. From such granules the secretory material may be funnelled into the extracellular space through a narrow channel (*curved arrows*). Case 1; **a** × 20,400; **b** × 13,450

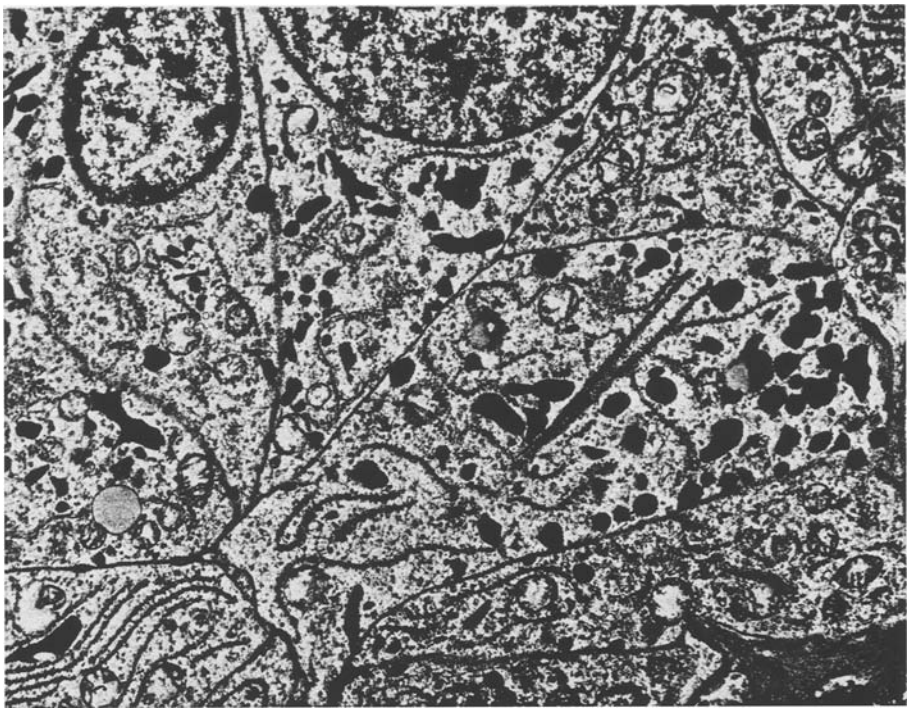

Fig. 4. The marked abnormality of granule formation, seen in the tumor of Case 4, is shown, ×7,150

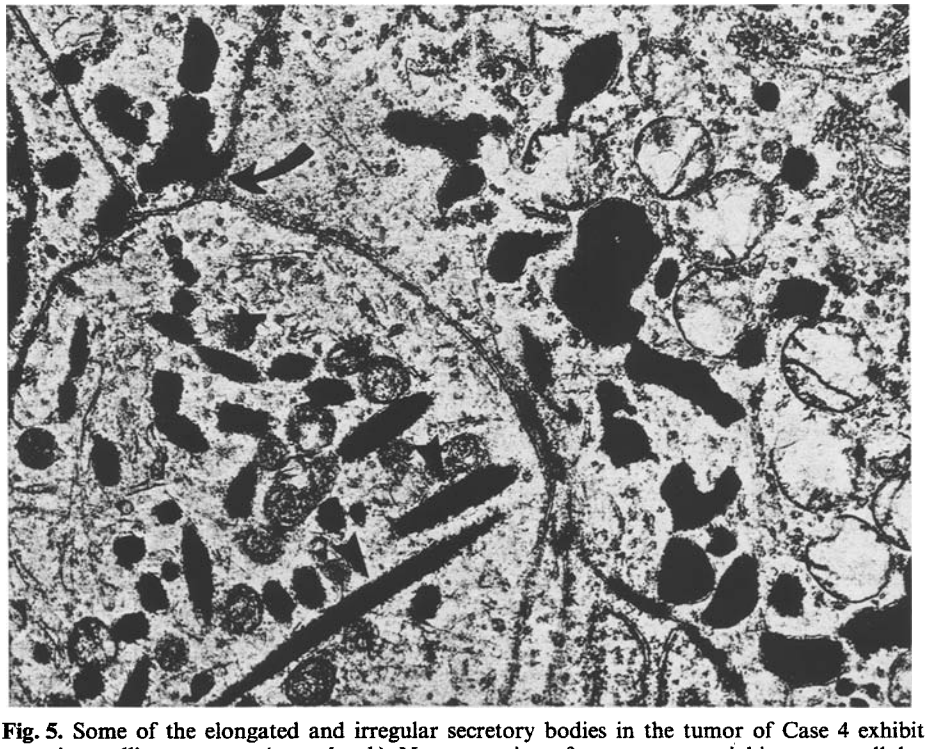

Fig. 5. Some of the elongated and irregular secretory bodies in the tumor of Case 4 exhibit a semicrystalline structure (*arrowheads*). Note extrusion of secretory material into extracellular space (*curved arrow*), ×14,200

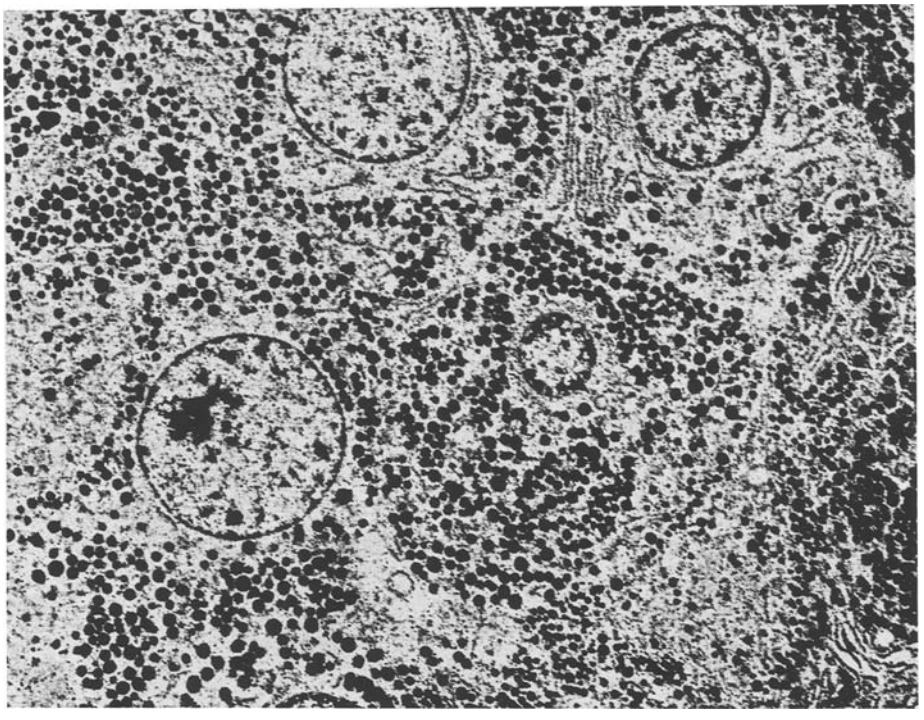

Fig. 6. Immunoelectron microscopy for growth hormone demonstrates positivity in every cell of this mammosomatotroph cell adenoma. Case 6, × 3,650

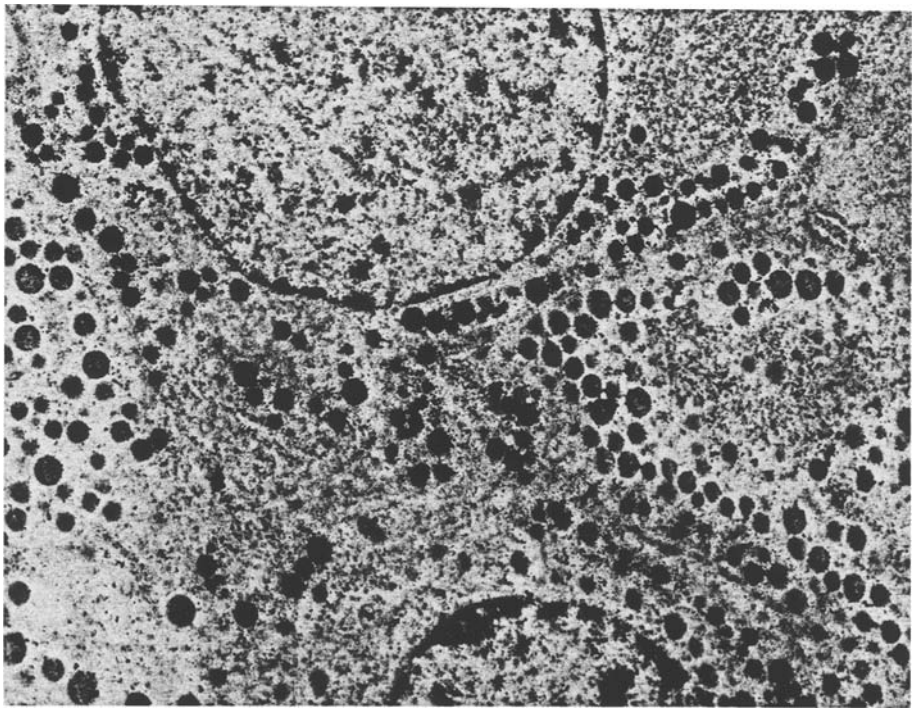

Fig. 7. Same case as Fig. 6. Immunoreactive prolactin is present in many cells. Numerous cells, however, contain few or no positive granules. Note that the positive and negative cells have the same fine structural appearance including size of secretory granules. Case 6, immunoelectron microscopy for prolactin, × 8,400

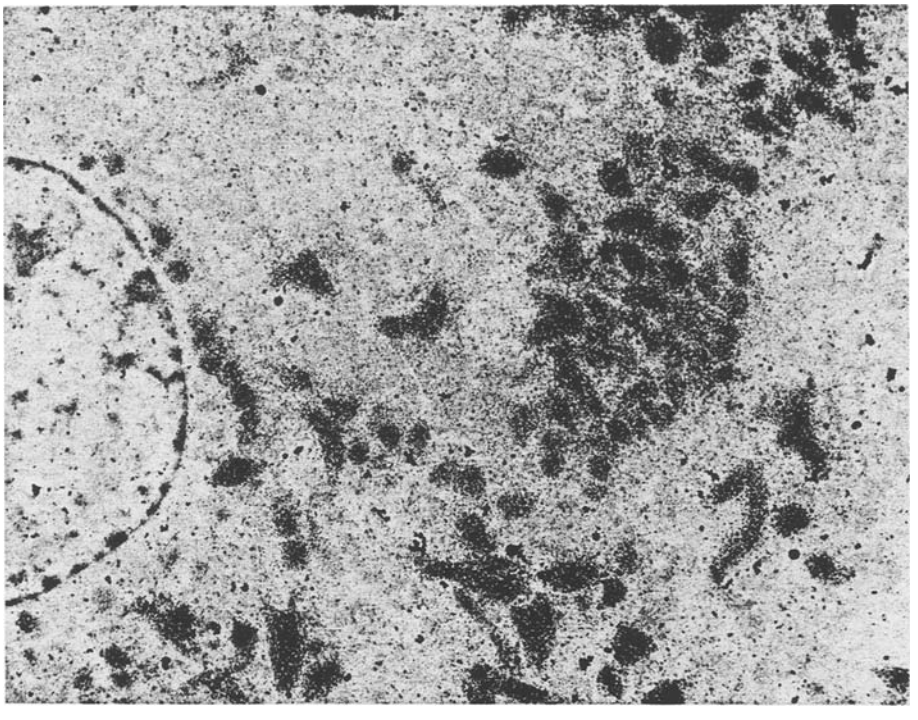

Fig. 8. Tumor of Case 4. Growth hormone is present in every cell, in both pleomorphic and spherical secretory granules. Immunoelectron microscopy for growth hormone, ×9,000

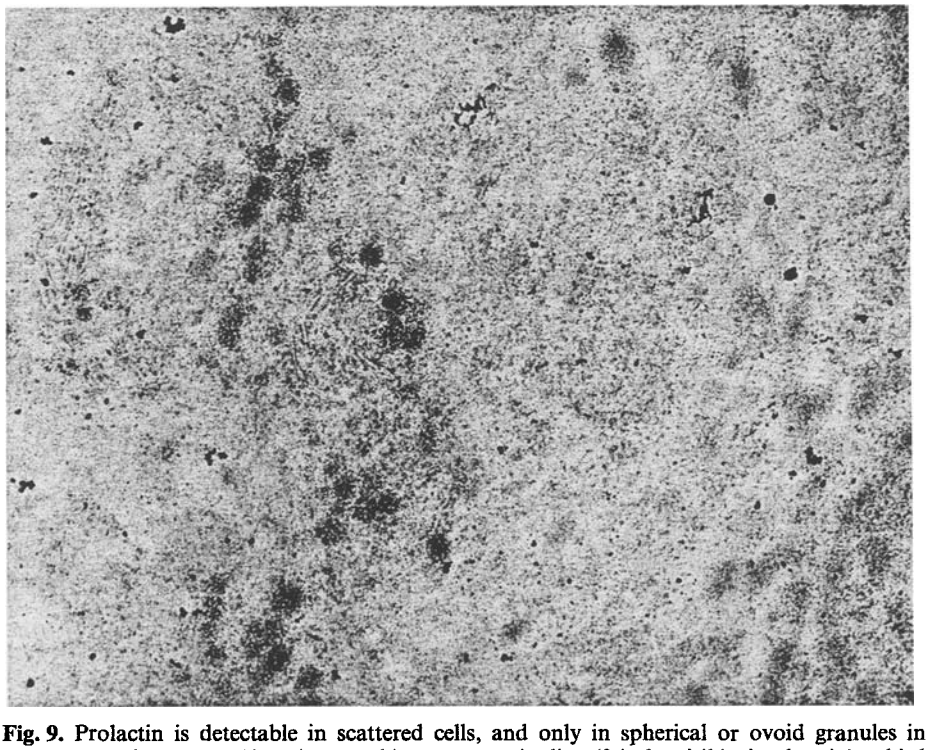

Fig. 9. Prolactin is detectable in scattered cells, and only in spherical or ovoid granules in the tumor of Case 4. The pleomorphic secretory bodies (faintly visible in the right third of the figure) are negative. Immunoelectron microscopy for prolactin, ×11,000

staining for immunoreactive prolactin were less numerous and were not present in every cell (Fig. 7). The marked morphologic abnormality of granule formation in case 4 helped to establish that 2 different granule populations may be present within the same cell. The pleomorphic and crystalline bodies and some of the spherical granules were positive for growth hormone (Fig. 8). Prolactin was demonstrated only in smaller (approx. 200–450 nm), predominantly spherical granules (Fig. 9). Neither growth hormone nor prolactin was conclusively demonstrated within the extracellular deposits of secretory material in any of the cases.

Discussion

The present study indicates that the 9 well differentiated monomorphous adenomas described here, consist of cells capable of producing both GH und PRL. To designate this tumor type which represents a morphologic entity, the name "mammosomatotroph cell adenoma" is proposed. It should be noted that Furth (1973) used the same term to specify certain estrogen-induced growth hormone and prolactin-producing tumors of the rat pituitary. Those bihormonal neoplasms, however, were bimorphous consisting of two cell populations. It appears that most human mammosomatotroph cell adenomas produce predominantly growth hormone and, with the exception of sporadic cases, prolactin is only a minor component. In most tumors, immunoelectron microscopy reveals growth hormone in every cell (although not in every granule), while prolactin content is variable.

The presence of two hormones in the tumor cells is associated with the morphologic heterogeneity of secretory granules. The majority of them have high electron density and closely fitted limiting membrane similar to those seen in growth hormone cell adenomas and prolactin-producing adenomas. The predominantly large granules with often asymmetrically placed, mottled core and conspicuous, irregular limiting membrane, appear only in mammosomatotroph cell adenomas. A prominent morphologic marker of this tumor type is the presence of extracellular deposits of secretory material. As opposed to the small, extruded granules of prolactin cell adenomas which dissipate quickly in the intercellular space, the electron density of large, extracellular deposits in mammosomatotroph adenomas seems to persist. Despite the use of optimally fixed and processed tissue for immunoelectron microscopy, we could not conclusively demonstrate hormones in the extracellular aggregates; the reason for that might be technical. Landolt and Rothenbühler (1978), who detected growth hormone in such deposits, claimed that the hormone was preserved only in tissue fixed in osmic acid.

The immunocytochemical results in case 4 strongly suggest that, at least in some cases, the two hormones are present in different granule populations. In this tumor, the large pleomorphic granules contained only growth hormone. This is concordant with our earlier observations that this type of abnormality of granule formation, although to a much lesser extent, occurs occasionally in densely granulated growth hormone cell adenomas.

Immunoreactive prolactin was seen only in spherical or slightly irregular granules measuring 200–450 nm, the predominant granule type in both normal and neoplastic prolactin cells (Pelletier et al. 1978; Horvath and Kovacs 1980).

The histogenesis of mammosomatotroph cell adenoma is not established. In our material, 54% of surgically-removed pituitary adenomas (330 tumors) derives from the "acidophil" cell line. Fifty-six of these, representing 3 morphologic types, are capable of producing both growth hormone and prolactin. The bimorphous mixed adenomas, consisting of growth hormone cells and prolactin cells, are the most common. The two other bihormonal adenoma types are monomorphous, probably originating from different cell types. The immature, aggressive acidophil stem cell adenoma is assumed to arise in the common precursor of growth hormone cells and prolactin cells (Horvath et al. 1981). In this neoplasm, the prolactin component is dominant. Growth hormone, although detectable in the tumors in most cases, is usually not elevated in the blood and acromegalic features are rare. Conversely, most patients with well differentiated mammosomatotroph cell adenoma have a history of slowly developing, typical acromegaly and the prolactin-secreting potential of the tumor may not be suspected unless the tissue is examined by immunohistochemistry and electron microscopy. It may well be that the cell type giving rise to these tumors is a separate mammosomatotroph cell. Such well differentiated, well granulated cells, containing both growth hormone and prolactin, are noted both in nontumorous human and rodent pituitaries (Horvath et al. 1979; Stratmann et al. 1974). It is also noteworthy that while acidophil stem cell adenoma occurs more commonly in women, there is a marked male preponderance in patients with mammosomatotroph cell adenoma.

As far as the clinical implications are concerned, the overwhelming majority of mammosomatotroph cell adenomas (or "growth hormone cell adenomas with extracellular growth hormone deposits;" Landolt and Rothenbühler 1978; Kameya et al. 1980; Trouillas et al. 1980) are indistinguishable from well-differentiated growth hormone cell adenomas associated with long-standing acromegaly, possibly associated with mild hyperprolactinemia. Uncommonly, tumors may also develop which secrete large amounts of prolactin with the overproduction of growth hormone being less pronounced. There is a remarkable similarity between our case 7 and Landolt and Rothenbühler's (1978) case 3, having such an adenoma. In such cases, the hyperprolactinemia and its sequelae may be more dominant than acromegaly. These neoplasms are partly chromophobic and reach a larger size than the majority of mammosomatotroph tumors. Thus, it appears that the growth pattern of these adenomas is similar to that of actively growing prolactin cell adenomas, rather than to that of well-differentiated and usually stationary growth hormone cell adenomas.

A fine structural investigation is essential in the diagnosis of mammosomatotroph cell adenoma. From the clinical point of view, its biological behavior is similar to that of well-differentiated growth hormone cell adenoma and mixed (growth hormone cell-prolactin cell) adenoma. As most of these tumors are well demarcated, intrasellar neoplasms, a high surgical cure rate for mammosomatotroph adenomas can be predicted.

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References

Furth J, Ueda G, Clifton KH (1973) The pathophysiology of pituitaries and their tumors. In: Busch H (ed) Methods in cancer research. Academic Press, pp 201–277

Horvath E, Kovacs K (1980) Pathology of the pituitary gland. In: Ezrin C, Horvath E, Kaufman B, Kovacs K, Weiss MH (eds) Pituitary diseases. CRC Press, Boca Raton, Fla., pp 1–83

Horvath E, Kovacs K, Singer W, Smyth HS, Killinger DW, Ezrin C, Weiss MH (1981) Acidophil stem cell adenoma of the human pituitary: clinicopathologic analysis of 15 cases. Cancer 47:761–771

Horvath E, Ryan N, McComb DJ, Kovacs K, Ezrin C (1979) Subcellular immunohistochemistry of the human pituitary. Proc Electr Micr Soc Am 37:226–227

Kameya T, Tsumuraya M, Adachi I, Abe K, Ichikizaki K, Toya S, Demura R (1980) Ultrastructure, immunohistochemistry and hormone release of pituitary adenomas in relation to prolactin production. Virchows Arch [Pathol Anat] 387:31–46

Kovacs K, Horvath E, Ryan N (1981) Immunocytology of the human pituitary. In: De Lellis RA (ed) Diagnostic Immunohistochemistry. Masson USA Inc, New York, NY, pp 17–35

Landolt AM, Rothenbühler V (1978) Extracellular growth hormone deposits in pituitary adenoma. Virchows Arch [Pathol Anat] 378:55–65

Moriarty GC, Garner LL (1977) Immunocytochemical studies of cells in the rat adenohypophysis containing both ACTH and FSH. Nature 265:356–358

Pelletier G, Robert F, Hardy J (1978) Identification of human anterior pituitary cells by immunoelectron microscopy. J Clin Endocrinol Metab 46:534–542

Stratmann IE, Ezrin C, Sellers EA (1974) Estrogen-induced transformation of somatotrophs into mammotrophs in the rat. Cell Tissue Res 152:229–238

Trouillas J, Girod C, Lhéritier M, Claustrat B, Dubois MP (1980) Morphological and biochemical relationships in 31 human pituitary adenomas with acromegaly. Virchows Arch [Pathol Anat] 389:127–142

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